SUPPLEMENTAL INFORMATION

TABLE I-A: *Amino Acid Mix Composition of Diet Used in this Study*

Amino Acid	Amino Acid Mix
Alanine	1.03
Arginine	1.06
Asparagine-H_2O	1.88
Cystine	0.42
Glutamic Acid	2.21
Glycine	0.57
Histidine-HCl-H_2O	0.57
Isoleucine	1.09
Leucine	1.53
Lysine-HCl	1.51
Methionine	0.58
Phenylalanine	0.99
Proline	0.72
Serine	1.32
Threonine	0.87
Tyrosine	0.24
Tryptophan	0.72
Valine	0.19
Total	15.91

[a] Both amino acid mixes were expressed in g/100 g diet. All amino acids were purchased from Ajinomoto, Tokyo, Japan.

TABLE I-B: *Composition of Diet Used in this Study*

Component	g/100 g diet
Amino Acid Mix	17.5
Vitamin Mix[a]	1
Mineral Mix[b]	5
Corn Oil[c]	5
Choline Chloride[d]	0.1
Sucrose[e]	23.47
Cornstarch[f]	47.93
Total	100

[a] Vitamin Supplement (TD USB Vitamin Supplement, Purina Test Diets): composition per kilogram of supplement: alpha-tocopherol, 1000 lU/g, 5.0 g; L-ascorbic acid, 45 g; choline chloride, 75.0 g; D-calcium pantothenate, 3.0 g; inositol, 5.0 g; menadione, 2.25 g; niacin, 4.5 g; p-aminobenzoic acid, 5.0 g;

pyridoxine HC1, 1.0 g; riboflavin, 1.0; thiamin HC1, 1.0; retinyl acetate, 900,000 units; ergocalciferol, 100,000 units; biotin, 20 mg; folic acid, 90 mg; vitamin B-12, 1.35 mg.
[b]Minerals (AIN76 Mineral Mixture, ICN Biochemicals) (g/kg): Calcium Phosphate Dibasic 500g; Sodium Chloride 74g; Potassium Citrate Monohydrate 220g; Potassium Sulfate 52g; Magnesium Oxide 24g, Manganese Carbonate (43-48%Mn) 3.5g; Ferric Citrate (16-17% Fe) 6g; Zinc Carbonate (70%ZnO) 1.6g; Cupric Carbonate (53-55%Cu) 0.3g; Potassium Iodate 0.01g; Sodium Selenite 0.01g; Chromium Potassium Sulfate 0.55g, Sucrose, finely powdered 118.0g.
[c]Mazola corn oil (Best Foods, Englewood Cliffs, NJ).
[d]Choline Chloride (Fisher): 50% in water
[e]Sucrose (Spreckles, San Francisco, CA)
[f]Cornstarch (National Starch and Chemical, Bridgewater, NJ): Melojel food grade cornstarch.

TABLE I-C: *Nitrogen, Total Protein, and Caloric Content Comparisons among Diets*

	Diet	NRC 1995
Nitrogen*	2.4	*2.4*
Protein	17.5	*15*
Caloric Content	4.0	*3.8 – 4.1*

* Nitrogen and Protein contents were expressed in g/ 100 g diet. Caloric content was expressed in kcal/ g diet.

TABLE II: *Total mitochondrial pool of amino acids from various species and organs*

SPECIES	ORGAN	FREE AMINO ACIDS (NMOL/MG PROTEIN)	REFERENCE
Rat	Liver	28.22 (29.39)*	This study
	Liver	96.00	[1]
	Liver	218.97	[2]
	Liver	464.8	[3]
	Liver	4.86	[4]
	Liver	68 to 104	[5]
	Liver	24.8	[6]
Rat	Brain	230	[1]
Mouse	Brain	30.2, 149.4	[6]
Sheep	Liver	102	[1]
	Brain	260	[1]
	Kidney	126	[1]
	Spleen	630	[1]
Ox	Heart	196	[1]
Pigeon	Breast muscle	54	[1]
	Liver	17.2	[4]

* The number between parentheses includes all metabolites listed in Table I excluding peptides (GSH and GSSG).

Comparison with other published reports on mitochondrial pool of amino acids

When the amino acid profile for rat liver mitochondria was compared to others published before, the main difference was that, to our knowledge, this is the first study that reports values for Asn, Cys, Pro, Trp, and Tau, whereas these compounds were not detected before because of either the method used (e.g., Asn detected as Asp) or the concentrations were below the detection limit [2-4]. In addition, we evaluated several other biologically relevant compounds, whose concentration was detected as negligible. These compounds were: β-Ala, cystathionine, δ-hydroxy-lysine, ethanolamine, γ-amino-butyric acid, hydroxy-Pro, 1- and 3-methyl-His, α-aminoadipic acid, α-aminobutyric acid, anserine, carnosine, citrulline, homocysteine, sarcosine, and phosphoethanolamine.

Significant reasons for the differences are the following: one, species-specific differences; two, time of sampling; and three, quality of mitochondria and methods used. In this study, male Sprague-Dawley rats were used whereas in [2] female Wistar rats (of about the same body weight and age as ours) and in [4] male Wistar rats (of 200-300 g body weight) were used. Although these strains share several similarities, it has also been reported of critical differences between them [7-9] and between sexes [10, 11]. On these reports quoted in Table II, no data on the time of euthanasia or light cycle period were reported making difficult to formulate a thorough comparison. For example, important diurnal variations in LOR and SDH activities had been reported which may produce fluctuations in Lys concentrations [12]. Finally, low RCRs or increased oxidative stress (reflected as an increase in oxidized thiols) might result on permeable mitochondrial membranes that allow an artifactual encapsulation of amino acids from the cytosol, resulting in concentrations that might reflect the putative gradient for these metabolites between compartments, i.e., cytosol and mitochondria. If outlier values are excluded (2-times above or half of our values; underlined numbers in Table II), our numbers were more similar to those reported by Baird [3]. However, this last report failed to measure concentrations for Arg, Asn, Cys, Gln, Pro, Trp, Orn and Tau, and relatively high (Glu, Lys, and Ser) or low (Met and Tyr) concentrations for other amino acids.

TABLE III: *Mitochondrial amino acid pools from various reports**

Amino acid	This study	Truman & Korner [2]	Baird [3]	Ferdinand et al. [4]
		(%)		
Ala	8.7	15.5	14.5	1.6
Arg	4.3	0.3	0.0	0.0
Asp	3.3	4.1	3.1	0.0
Asn	4.1	ND	ND	0.0
Cys	0.3	0.0	0.0	0.6†
Glu	1.1	15.2	3.9	6.3
Gln	4.0	ND	ND	1.6
Gly	4.1	24.6	8.2	3.9
His	2.2	4.8	2.0	1.7
Ile	6.3	1.0	5.5	0.9
Leu	14.2	2.1	9.0	1.7
Lys	8.4	7.3	17.7	0.0
Met	4.6	0.8	1.2	0.9
Phe	6.9	0.9	3.9	0.5
Pro	0.7	0.0	0.0	0.0
Ser	6.0	17.6	13.3	3.3
Thr	4.4	3.2	6.3	1.4
Trp	1.0	ND	0.0	0.0
Tyr	4.2	1.3	0.8	1.3
Val	4.9	1.3	10.6	9.9
EAA/NEAA	*1.1*	*0.6*	*1.3*	*1.0*

Others				
GSH	2.6	ND	ND	22.9‡
GSSG	0.2	ND	ND	ND
Tau	0.5	ND	ND	39.6
Orn	3.3	ND	ND	0.3
Citrulline	0.0	ND	ND	ND
Anserine	0.0	ND	ND	ND
Carnosine	0.0	ND	ND	ND
Sarcosine	0.0	ND	ND	0.5
1-Me-His	0.0	ND	ND	1.1
3-Me-His	0.0	ND	ND	0.0
Hydroxy-Lys	0.0	ND	ND	0.2
L-α-amino-butyrate	0.0	ND	ND	0.0

*Data for columns three, four, and five were taken from [2], [3], and [4], respectively. Data were expressed in percentages using 1 mg mitochondria dry weight/2 mg mitochondrial protein (calculated using numbers from [6] and [2]). ND, not determined, whereas 0.00 indicates below the detection limit. Underlined numbers are located outside the range of 2.5 x our values.

† This value represents the amount of cystine determined by [4] multiplied by 2 to represent Cys. If the value of cysteic acid reported in [4] were added to this value, its percentage would be 12.78%.

‡ This value in [4] represents GSH + GSSG.

FIGURE 1 *Correlation of mitochondrial tRNA levels and free amino acids in mitochondria*
The levels of tRNA were obtained from [13] and those of the amino acids were the experimental numbers obtained in this study.

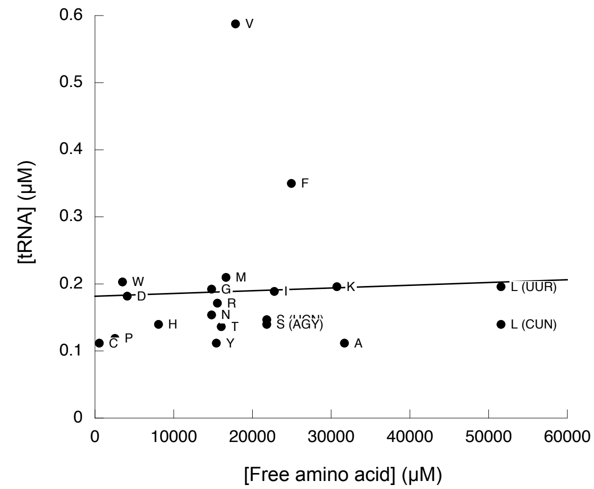


FIGURE 2 *Correlation of free amino acid concentrations in mitochondria and codon usage or the frequency of its usage in translation*
The amino acid concentrations were those experimentally determined and expressed in concentration considering the matrix volume in mitochondria[1]. The relative codon usage in translation was taken from [14]. The amino acids are indicated by their single letter amino acid code.

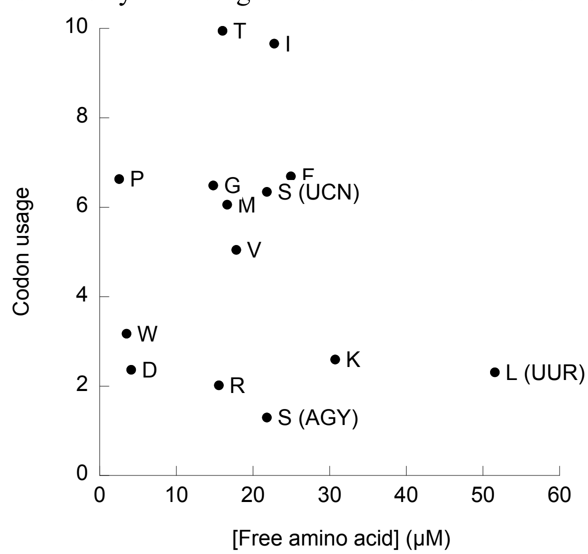


FIGURE 3 *Correlation of mitochondrial mRNA half-life and correlation coefficients between free amino acid concentrations and amino acid composition of mitochondrial subunits*
The half-lives of mitochondrial transcripts were taken from HeLa cells [15]. The correlation coefficients were calculated by plotting the amino acid composition of each subunit vs. the free amino acid concentration in the matrix.

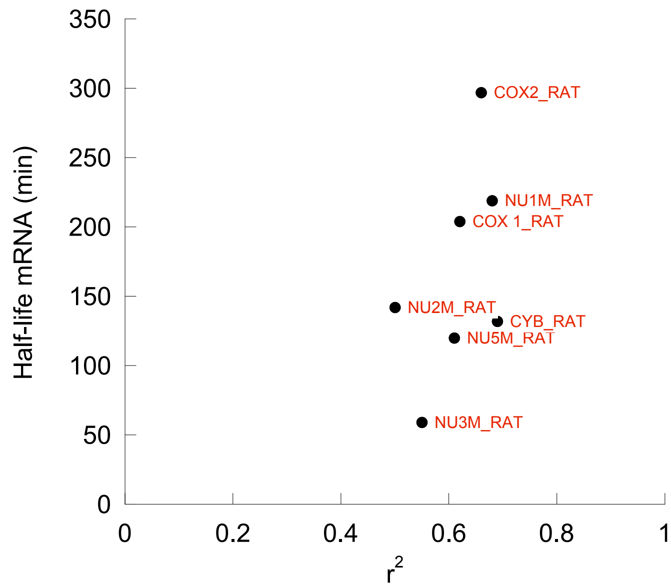
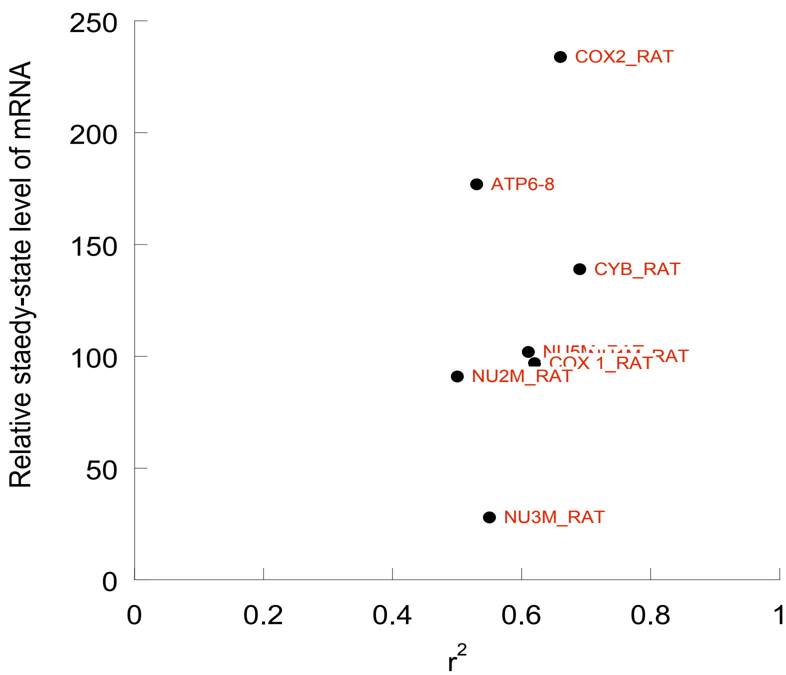


FIGURE 4 *Correlation of steady-state concentrations of mitochondrial transcripts and free amino acid concentrations*
The steady-state concentrations of mRNA for mtDNA-encoded proteins were taken from [15]. The correlation coefficients were obtained as described under Fig. 3.



REFERENCES FOR SUPPLEMENT

1 Alberti, K. G. and Bartley, W. (1963) The production of amino acids by cell fractions, particularly rat-liver mitochondria. Biochem J. **87**, 104-114

2 Truman, D. E. and Korner, A. (1962) Incorporation of amino acids into the protein of isolated mitochondria. A search for optimum conditions and a relationship to oxidative phosphorylation. Biochem J. **83**, 588-596

3 Baird, G. D. (1964) The Release of Amino Acids from Rat-Liver Mitochondrial Extract. Biochim Biophys Acta. **93**, 293-303

4 Ferdinand, W., Bartley, W. and Broomhead, V. (1973) Amino acid production in isolated rat liver mitochondria. Biochem J. **134**, 431-436

5 Alberti, K. G. and Bartley, W. (1965) Further Observations on the Production of Amino Acids by Rat-Liver Mitochondria and Other Subcellular Fractions. Biochem J. **95**, 641-656

6 Bellamy, D. (1962) The endogenous citric acid-cycle intermediates and amino acids of mitochondria. Biochem J. **82**, 218-224

7 Hlavacova, N., Bakos, J. and Jezova, D. (2006) Differences in home cage behavior and endocrine parametres in rats of four strains. Endocr Regul. **40**, 113-118

8 Walberer, M., Stolz, E., Muller, C., Friedrich, C., Rottger, C., Blaes, F., Kaps, M., Fisher, M., Bachmann, G. and Gerriets, T. (2006) Experimental stroke: ischaemic lesion volume and oedema formation differ among rat strains (a comparison between Wistar and Sprague-Dawley rats using MRI). Lab Anim. **40**, 1-8

9 Geis, R. B., Diel, P., Degen, G. H. and Vollmer, G. (2005) Effects of genistein on the expression of hepatic genes in two rat strains (Sprague-Dawley and Wistar). Toxicol Lett. **157**, 21-29

10 Terner, J. M., Barrett, A. C., Cook, C. D. and Picker, M. J. (2003) Sex differences in (-)-pentazocine antinociception: comparison to morphine and spiradoline in four rat strains using a thermal nociceptive assay. Behav Pharmacol. **14**, 77-85

11 Terner, J. M., Lomas, L. M., Smith, E. S., Barrett, A. C. and Picker, M. J. (2003) Pharmacogenetic analysis of sex differences in opioid antinociception in rats. Pain. **106**, 381-391

12 Blemings, K. P., Crenshaw, T. D. and Benevenga, N. J. (1998) Mitochondrial lysine uptake limits hepatic lysine oxidation in rats fed diets containing 5, 20 or 60% casein. J Nutr. **128**, 2427-2434

13 King, M. P. and Attardi, G. (1993) Post-transcriptional regulation of the steady-state levels of mitochondrial tRNAs in HeLa cells. J Biol Chem. **268**, 10228-10237

14 Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J., Staden, R. and Young, I. G. (1981) Sequence and organization of the human mitochondrial genome. Nature. **290**, 457-465

15 Piechota, J., Tomecki, R., Gewartowski, K., Szczesny, R., Dmochowska, A., Kudla, M., Dybczynska, L., Stepien, P. P. and Bartnik, E. (2006) Differential stability of mitochondrial mRNA in HeLa cells. Acta Biochim Pol. **53**, 157-168